THE VALUE OF PRESUMPTIVE TESTS FOR BACILLUS COLI BASED ON THE ROUTINE USE OF LACTOSE BILE AND LACTOSE BROTH

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Previous to 1906, glucose broth was generally used in the presumptive test for Bacillus coli. As a result of the work of Jackson (1906, 1907), Weston and Tarbett (1907), Sawin (1907), and others (Hale and Melia, 1910), lactose peptone bile was substituted for glucose broth by the American Public Health Association in the 1912 Standard Methods of Water Analysis. Doubt was, however, thrown upon the accuracy of the results obtained by the use of lactose bile by Jordan (1913) and Obst (1916), which resulted in another change by the American Public Health Association in 1917, lactose broth being substituted for lactose bile. The authors cited showed that lactose bile not only inhibited organisms outside of the typhoid-colon group, but that it also had an inhibitive action on members of this group itself.

In this laboratory, both bile and broth are used for the detection of B. coli in tap waters; and the present study is based on examinations of 1899 samples of tap water showing fermentation in bile, or broth, or both, from the forty-six city surface supplies in Kansas, of which weekly tests are made. These cover the period from July 1, 1918, to May 15, 1919.

The bile used is prepared by diluting oxgall 50 per cent with distilled water and adding 1 per cent lactose and 1 per cent peptone. Both large and small culture tubes with inverted vial fermentation tubes are filled,—the large tubes with 15 cc. of medium and the small ones with 7 cc.
The broth, which is tubed in the large fermentation tubes, contains 0.3 per cent of Liebig’s Beef Extract, 1 per cent peptone and 1 per cent lactose, and is adjusted to a point neutral to phenolphthalein.

Endo plates are made of 3 per cent agar with 1 per cent lactose and 0.37 per cent fuchsin decolorized with 5 per cent sodium sulphite. The reaction is adjusted to 0.5 per cent acid to phenolphthalein. This amount of fuchsin gives a rapid and brilliant coloration with acid formers, and a good metallic sheen on the colonies of *B. coli*.

Each sample is inoculated as follows: 1 cc. into each of five small bile tubes, 10 cc. into one large bile tube, 10 cc. into each of three large broth tubes.

These are incubated at 37° and after twenty-four hours, the cultures are read; those showing 10 per cent or more gas in the fermentation tube, or showing active fermentation, being streaked on Endo plates for confirmation, and the remaining cultures being returned to the incubator until forty-eight hours have elapsed. They are then read once more, positive cultures confirmed, and all discarded. Endo plates are incubated about twenty hours.

In recording results, twenty-four hour readings are marked in red ink, forty-eight hour readings in blue ink, with + and − to show presence or absence of gas. To indicate a 10 cc. sample, a circle is placed around the symbol. Bile results are always recorded above broth to avoid confusion.

Table 1 shows the relationship between confirmatory tests and the presence of gas in one or both of the bile and broth media. These results have been studied in an effort to learn if the simultaneous use of the two media might make confirmation unnecessary.

When both media are used and both show fermentation, 75.05 per cent confirm in either bile, broth, or both media. When bile alone shows fermentation, which is in a very small number of samples, 34.42 per cent confirm, as opposed to 25.20 per cent when broth alone is positive, which occurs in ten times as many cases. According to these results when both media are used in the presumptive test, there are fewer mistakes than when either is used separately.
The next step was to examine the results to determine if the number of broth tubes showing fermentation is any indication of the presence of typical *B. coli*.

It will be noted from table 2 that when all three broth tubes are positive, the percentage confirmed is above that calculated.
for the whole number of tests with both media fermenting, the average in the former being 81.18 per cent and in the latter 75.05 per cent. When only two of the three tubes are positive, 60.61 per cent confirm, and when only one ferments 54.95 per cent confirm. If both media are used and there is fermentation in any of the bile and all of the broth tubes, fewer unconfirmed presumptive tests will be obtained than in the cases where the number of tubes reacting positively is one or two.

**TABLE 3**

<table>
<thead>
<tr>
<th></th>
<th>NUMBER SAMPLES BILE AND BROTH NUMBER SAMPLES BILE AND BROTH</th>
<th>PER CENT SAMPLES BILE AND BROTH</th>
<th>NUMBER SAMPLES BILE ONLY CONFIRMING</th>
<th>PER CENT SAMPLES BILE ONLY CONFIRMING</th>
<th>NUMBER SAMPLES BROTH ONLY CONFIRMING</th>
<th>PER CENT SAMPLES BROTH ONLY CONFIRMING</th>
<th>TOTAL PER CENT CONFIRMING</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bile, +, Broth, +, 24 hours</td>
<td>387</td>
<td>362</td>
<td>93.54</td>
<td>6</td>
<td>1.55</td>
<td>10</td>
<td>2.58</td>
</tr>
<tr>
<td>Bile, +, Broth, -, 24 hours</td>
<td>8</td>
<td>3</td>
<td>37.50</td>
<td></td>
<td></td>
<td></td>
<td>37.50</td>
</tr>
<tr>
<td>Bile, -, Broth, +, 24 hours</td>
<td>42</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>54.76</td>
</tr>
<tr>
<td>Bile, +, Broth, +, 48 hours</td>
<td>82</td>
<td>50</td>
<td>69.75</td>
<td>7</td>
<td>8.53</td>
<td>4</td>
<td>4.89</td>
</tr>
<tr>
<td>Bile, +, Broth, -, 48 hours</td>
<td>124</td>
<td>86</td>
<td>69.35</td>
<td>3</td>
<td>2.43</td>
<td>24</td>
<td>19.35</td>
</tr>
</tbody>
</table>

Another point for consideration is whether or not this same conclusion applies when the broth only is positive. By comparing the last three percentages in the preceding table this is seen to be the case, as when three tubes are positive there is 29.26 per cent confirmation, when two are positive, 22.78 per cent when only one is positive, 22.49 per cent, respectively.

A study of the time factor was next made and summarized in table 3.

There is no need for confirmation when both bile and broth show fermentation in twenty-four hours, as 97.67 per cent of
the samples so reacting proved to be positive in either bile, broth, or both media. If either bile or broth is positive in twenty-four hours and the other in forty-eight hours, there is a higher percentage of samples containing \( B. \) \( \text{coli} \) than when both bile and broth are positive only after forty-eight hours, as is shown by 83.17 per cent of confirmation with bile positive in twenty-four hours and broth in forty-eight hours, and 91.13 per cent with broth positive in twenty-four hours and bile in forty-eight hours.

This would indicate that while bile is fermented more slowly than broth, yet in dealing with contaminated waters it is just as accurate.

The statistics of these results on 1899 samples of surface waters examined for pollution, where both bile and broth are used simultaneously, would suggest the following conclusions:

1. If there is fermentation in both bile and broth tubes, the presumptive test is reliable in 75.05 per cent of all cases considered.
2. If all three broth tubes, as well as the bile, are positive, a greater percentage of tests are confirmed as \( B. \) \( \text{coli} \) than is the case when only one or two tubes out of three are positive.
3. When broth alone is positive the water in 70 per cent to 78 per cent of cases is proved not to contain \( B. \) \( \text{coli} \).
4. Samples with both media positive in twenty-four hours contain \( B. \) \( \text{coli} \) in 97.67 per cent of cases and therefore do not need confirmation.

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